15) OECD 211-OPPTS 850.1350, Chronic toxicity test (daphnia magna), 454A-130

(2)

T-7485
PFBS
OECD 211
OPPTS 850.13

PFBS:
A SEMI-STATIC LIFE-CYCLE TOXICITY TEST
WITH THE CLADOCERAN (*Daphnia magna*)

FINAL REPORT

SANITIZED

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-130
3M Environmental Lab Project No. E00-1429

DEC 09 2003

U.S. Environmental Protection Agency
Series 850 – Ecological Effects
OPPTS Number 850.1300

OECD Guideline 211

AUTHORS:

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STUDY INITIATION DATE: October 6, 2000

STUDY COMPLETION DATE: April 11, 2001

Submitted to

3M Corporation
Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, Minnesota 55144

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

SANITIZED

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFBS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-130

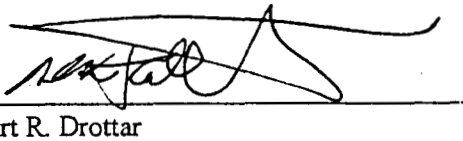
STUDY COMPLETION: April 11, 2001

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984, with the following exceptions:

The test substance was not characterized in compliance with Good Laboratory Practices prior to its use in the study. However, subsequent GLP compliant characterization resulted in a purity similar to the original characterization purity.

The stability of the test substance under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:



Kurt R. Drottar
Senior Biologist

4/11/01
DATE

SPONSOR'S REPRESENTATIVE:

_____

4/17/01
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	February 7, 2001	February 7, 2001	February 9, 2001
Analytical Sample Preparation	February 9, 2001	February 9, 2001	February 14, 2001
Exposure Initiation, Offspring	February 21, 2001	February 21, 2001	February 22, 2001
Observations, Length Measurements and Offspring Counts	February 28, 2001	February 28, 2001	March 2, 2001
Analytical Sampling	February 28, 2001	February 28, 2001	March 2, 2001
Biological Data and Draft Report	March 21 – 23 and 26, 2001	March 26, 2001	March 27, 2001
Analytical Data and Draft Report	March 26 and 29, 2001	March 29, 2001	March 29, 2001
Final Report	April 10 and 11, 2001	April 11, 2001	April, 11, 2001

Kimberly A. Hoxter

Kimberly A. Hoxter
Quality Assurance Representative

4-11-01
DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFBS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-130

STUDY DIRECTOR:

Kurt R. Drottar
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4/11/01
DATE

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Director, Aquatic Toxicology and
Non-Target Plants

DATE 4/11/01

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SUMMARY

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	454A-130
TEST SUBSTANCE:	Perfluorobutanesulfonate, Potassium Salt (PFBS)
STUDY:	PFBS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)
NOMINAL TEST CONCENTRATIONS	Negative Control, 63, 125, 250, 500, 1000 and 2000 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, 60, 121, 247, 502, 995 and 1876 mg a.i./L
TEST DATES:	Experimental Start – February 7, 2001 Biological Termination – February 28, 2001 Experimental Termination – March 2, 2001
LENGTH OF TEST:	21 Days

TEST ORGANISM:	Neonate Cladocerans (*Daphnia magna*)
SOURCE OF TEST ORGANISMS:	Wildlife International, Ltd. cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	Juveniles < 24 hours old

NOEC:	502 mg a.i./L
LOEC:	995 mg a.i./L
MATC:	707 mg a.i./L

INTRODUCTION

This study was conducted by Wildlife International Ltd. for 3M Corporation at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from February 7, 2001 to February 28, 2001. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454A-130 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to determine the effects of Perfluorobutanesulfonate, Potassium Salt (PFBS) on the survival, growth and reproduction of the cladoceran (*Daphnia magna*) during a 21-day exposure period under semi-static test conditions.

EXPERIMENTAL DESIGN

Daphnids were exposed to a geometric series of six test concentrations and a negative (dilution water) control. Ten replicate test chambers were maintained in each treatment and control group with one daphnid in each test chamber. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of an acute toxicity test. Nominal test concentrations were 63, 125, 250, 500, 1000 and 2000 mg active ingredient (a.i.)/L. Mean measured concentrations were determined from new and corresponding old solutions during each week of the test. Daphnids were transferred to new solutions every Monday, Wednesday and Friday during the test.

First-generation daphnids were observed daily for survival, the onset of reproduction, and clinical signs of toxicity. With the onset of reproduction, the number of second-generation neonates were counted (number of live neonates, number of dead or immobile neonates and number of aborted eggs) every Monday, Wednesday and Friday during the test. If available, twenty second-generation neonates from each treatment and the control were exposed under static test conditions for 48 hours. At test termination, the length and dry weight of each first-generation daphnid was determined. The no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) were determined by examination of the mortality, growth and reproduction data. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and LOEC.

MATERIALS AND METHODS

The study was conducted based on the procedures outlined in the protocol, "PFBS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)". The protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1300 (1); OECD Guideline 211: *Daphnia magna* Reproduction Test (2); *Standard Evaluation Procedure, Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test* (3); and ASTM Standard E 1193-96, *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna* (4)...

Test Substance

The test substance was received from 3M Corporation on June 28, 2000 and was assigned Wildlife International, Ltd. identification number 5292. The test substance, a white powder, was identified as Potassium Perfluoro Butane Sulfonate, AKA Developmental Product, AKA PFBS, lot 2, CAS Number 29420-49-3. Information provided by the Sponsor indicated a purity of 97.90%. A subsequent revision of the certificate of analysis indicated a purity of 97.3% and an Expiration/Reassessment Date of January 17, 2002. The test substance was stored at ambient room temperature.

Preparation of Test Concentrations

Nominal test concentrations were 63, 125, 250, 500, 1000 and 2000 mg a.i./L. A 4-L primary stock solution was prepared by adding the test substance to UV sterilized dilution water at a concentration of 2000 mg a.i./L. The test solution was stirred with an electric stainless steel top-down mixer until all the test substance was dissolved. The primary stock solution was proportionally diluted with UV sterilized dilution water to prepare 2-L of the other five test concentrations. All dilutions were inverted to mix. After mixing, 200 mL of test solution was added to the 10 replicate test chambers for each treatment group. New solutions were prepared for each renewal day. After mixing, all test solutions appeared clear and colorless. All test concentrations were adjusted for the original reported purity of the active ingredient in the test substance (97.9%).

Test Organism

The cladoceran, *Daphnia magna*, was selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates and were selected for use in the test based upon

past history of use and ease of culturing in the laboratory. Daphnid neonates used in the test were less than 24-hours old and were obtained from cultures maintained by Wildlife International, Ltd., Easton, Maryland.

Adult daphnids were cultured at approximately the same temperature as used during the test in Wildlife International, Ltd. well water. Daphnids in the cultures were held for at least 15 days prior to collection of the juveniles for testing. The adults showed no signs of disease or stress during the holding period. During the 14-day holding period preceding the test, water temperatures ranged from 19.8 to 20.1°C. The pH of the water ranged from 8.1 to 8.3 and dissolved oxygen ranged from 8.3 to 8.6 mg/L. Instrumentation and methods used for water measurements are described in the *Environmental Conditions* section of this report.

Neonate daphnids (<24 hours old) were obtained for testing from four individual adult daphnids. At test initiation, the juvenile daphnids were collected from the cultures and placed in glass beakers one to two at a time until each beaker contained 10 daphnids. The beakers were then impartially assigned to treatment groups. One daphnid was then transferred to each test chamber using a wide-bore pipet underneath the air/water interface. Daphnids in the cultures and those used in the test were fed a mixture of yeast, Cerophyll®, and trout chow (YCT, 1800 mg TSS/L), as well as a suspension of the freshwater green alga, *Selenastrum capricornutum* (approximately 3.5×10^7 cells/mL). During the test, each test chamber was fed 0.5 mL of YCT and 1.0 mL of *Selenastrum* once daily.

Test Apparatus

Test chambers were 250-mL plastic (Nalgene®) beakers containing 200 mL of test solution. The depth of test solution in a representative test chamber was approximately 5 cm. Test chambers were indiscriminately positioned by treatment group in an environmental chamber set to maintain a temperature of 20± 1°C. The test chambers were labelled with the project number, test concentration and replicate.

Dilution Water

The water used for culturing and testing was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity, and pH of the well water during the four-week period immediately preceding the test are presented in Appendix 1.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to use, the water again was filtered to 0.45 µm and passed through a UV sterilizer to remove microorganisms and fine particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International, Ltd. are presented in Appendix 2.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity was measured at test initiation and at weekly intervals thereafter with a SPER Scientific Ltd. light meter. Light intensity ranged from 357 to 512 lux at the surface of the water (Negative Control, Replicate A).

Temperature was measured in alternating replicates of each treatment and the control group at test initiation, in old and new test solutions on renewal days, and at test termination using a liquid-in-glass thermometer. Temperature was also measured continuously in one negative control replicate using a Fulscope ER/C Recorder. Recorder measurements were verified with a liquid-in-glass thermometer prior to test initiation and at least weekly thereafter. The target test temperature was 20± 1°C. Dissolved oxygen was measured daily in alternating replicates during the first seven days of the test, in old and new solutions on renewal days, and at test termination. Measurements of pH were made in alternating replicates at test initiation, in old and new solutions on renewal days and at test termination. Hardness, alkalinity and specific conductance were measured in water samples collected from alternating replicates of the negative control and the highest test concentration at test initiation and at approximately weekly intervals thereafter (new solutions).

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter.

Hardness and alkalinity measurements were made by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (5).

Biological Observations and Measurements

The first generation daphnids were observed daily during the test for survival, the onset of reproduction, and clinical signs of toxicity. The criteria for death included white opaque coloration, lack of movement of appendages, absence of heartbeat and lack of response to gentle prodding. Immobilization was defined as lack of movement except for minor activity of the appendages. The presence of eggs in the brood pouch, aborted eggs, males or ephippia were also recorded.

With the onset of reproduction, the number of second-generation neonates were counted (number of live neonates, number of dead or immobile neonates and number of aborted eggs) and recorded every Monday, Wednesday and Friday during the test. At each observation period, the live first-generation daphnids were retained and the second-generation daphnids were discarded. On Day 14, the second-generation neonates were collected and used for the second-generation acute exposure. At test termination, the length (measured from the apex of the head to the base of the spine) and dry weight were determined for each surviving first-generation daphnid. The individual daphnids were removed from the test chambers and placed into tared aluminum drying pans. The length of each daphnid was determined using a caliper. The pans were then placed in a drying oven at approximately 60°C for approximately 46 hours. The pans were removed from the drying oven and placed in a desiccator to cool. When cool, the pans were weighed using a Mettler AE-240 analytical balance.

Statistical Analyses

Statistical analyses were performed on survival of first-generation daphnids, the number of live young and the length and dry weight of the surviving first-generation daphnids. Survival data were analyzed using Fisher's exact test. Treatment groups with significant survival effects were excluded from the analyses of reproduction and growth. Reproduction and growth (length and dry weight) data were evaluated for normality and homogeneity of variance using the chi-square test and Bartlett's test, respectively. If the data met the assumptions of normality and homogeneity, the Bonferroni t-test was used to identify treatment groups that were statistically reduced in comparison to the negative control ($p \leq 0.05$). If the data failed the assumptions of normality or homogeneity, transformations were applied to the data in an attempt to correct

the condition. If transformations failed, Wilcoxon's rank sum test with the Bonferroni adjustment was used to evaluate the data ($p \leq 0.05$). All statistical tests were performed on a personal computer using TOXSTAT Version 3.5 (6) statistical software.

Analytical Chemistry

Water samples were collected from new and old solutions each week during the test. Duplicate samples of new solutions were collected from the freshly prepared solutions. Duplicate samples of the old solutions were collected from a composite of the remaining replicate test chambers. Samples were collected in plastic scintillation vials and were analyzed as soon as possible without storage. Analytical procedures used in the analysis of the samples are provided in Appendix 3.

RESULTS AND DISCUSSION

Verification of Test Concentrations

Results of analyses to measure concentrations of PFBS in water samples collected during the test are presented in Table 1 and the analytical chemistry report (Appendix 3). Nominal concentrations selected for use in the study were 63, 125, 250, 500, 1000 and 2000 mg a.i./L. Measured values of new (freshly prepared) samples ranged from 85 to 103% of nominal values, whereas measured values from the old solutions ranged from 86 to 106% of nominal values. Consequently, PFBS was stable throughout the renewal periods. When measured concentrations of samples collected during each week of the test were averaged, the mean measured concentrations were 60, 121, 247, 502, 995 and 1876 mg a.i./L, which represented 95, 97, 99, 100, 100 and 94% of nominal, respectively. The results of the study were based on mean measured concentrations.

Physical and Chemical Measurements of Water

Measurements of temperature, dissolved oxygen and pH are presented in Tables 2, 3 and 4, respectively. Manual water temperature measurements were within the range of 20±1°C established for the test. However, there were two instances when the continuous temperature monitor reached 18°C. This occurred during renewal of test solutions and was probably the result of the temperature probe being exposed to air. Dissolved oxygen concentrations remained ≥7.9 mg/L (87% of saturation) throughout the test. Measurements of pH ranged from 7.9 to 8.7.

Measurements of specific conductance, hardness and alkalinity in the negative control and the highest treatment group are presented in Table 5. No apparent differences were observed for hardness or alkalinity throughout the exposure period. However, conductivity was elevated in the 1876 mg a.i./L treatment group in comparison to the negative control.

Survival and Biological Observations

A summary of the results of the observations of mortality and sublethal signs of toxicity for first-generation daphnids are shown in Table 6. Daily observations are provided in Appendix 4. After 21 days of exposure, survival in the negative control group was 90%. Survival in the 60, 121, 247, 502, 995 and 1876 mg a.i./L treatment groups was 100, 100, 100, 100, 70 and 20%, respectively. Fisher's exact test showed that survival was significantly different in the 1876 mg a.i./L treatment group in comparison to the negative control ($p \leq 0.05$).

Reproduction

A summary of the mean number of live young produced by the first-generation daphnids is presented in Table 7. A complete listing of the number of neonates produced is provided in Appendix 5. Daphnids in the control and PFBS treatment groups ≤995 mg a.i./L started producing neonates on Day 8. There were no observations of aborted eggs in any treatment group. Reproduction averaged 86 young per adult in the negative control. Reproduction in the 60, 121, 247, 502 and 995 mg a.i./L treatment groups averaged 173, 149, 167, 89 and 43 young per adult, respectively. The Bonferroni t-test showed that reproduction was significantly reduced in the 995 mg a.i./L treatment group in comparison to the negative control ($p \leq 0.05$). The 1876 mg a.i./L treatment group was not included in the statistical analysis of reproduction or growth data due to a statistically significant effect on survival.

Growth

A summary of the mean lengths and mean dry weights of the surviving first-generation daphnids are presented in Table 8. Individual measurements are provided in Appendix 6. The mean length and dry weight of first-generation daphnids in the negative control was 4.18 mm and 0.80 mg, respectively. Wilcoxon's rank sum test showed that mean length was significantly reduced in the 995 mg a.i./L treatment group in comparison to the negative control ($p \leq 0.05$).

Second-Generation Acute Exposure

The results of the second-generation acute exposure are presented in Table 9. After 48-hours of exposure, survival in the negative control was 100%. Survival in the 60, 121, 247, 502 and 995 mg a.i./L treatment groups was 100, 100, 100, 100 and 100%, respectively. A second-generation acute exposure was not performed for the 1876 mg a.i./L treatment group due to a lack of neonates.

CONCLUSIONS

There were no adverse effects on survival, reproduction or growth of *Daphnia magna* exposed to PFBS at concentrations ≤502 mg a.i./L. *Daphnia magna* exposed to 1876 mg a.i./L had significantly reduced survival. *Daphnia magna* exposed to 995 mg a.i./L had significantly reduced reproduction and length. Consequently, the LOEC for this study, based on reproduction and length was 995 mg a.i./L. The NOEC was 502 mg a.i./L and the MATC was calculated to be 707 mg a.i./L. The results of the second-generation acute exposure indicated a NOEC of 995 mg a.i./L.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1300: *Daphnia Chronic Toxicity Test.*

2 **OECD Guideline for Testing of Chemicals**. 1997. Guideline 211: *Daphnia magna Reproduction Test.*

3 **U.S. Environmental Protection Agency.** 1986. *Standard Evaluation Procedure, Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test.* Hazard Evaluation Division. Office of Pesticide Programs, EPA 540/9-86-141.

4 **American Society for Testing and Materials.** 1997. *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna*, ASTM E 1193-96. Philadelphia, PA.

5 **APHA, AWWA, WPCF.** 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American water Works Association. Water Pollution Control Federation, New York.

6 **West, Inc. and D.D. Gulley.** 1996. TOXSTAT, Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

Table 1

Summary of Analytical Chemistry Data

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Nominal Concentration (mg a.i./L)	Day 0 (New)	Day 2 (New)	Day 5 (Old)	Day 9 (New)	Day 12 (Old)	Day 16 (New)	Day 19 (Old)	Day 21 (Old)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal
Negative Control	<LOQ[1]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	<LOQ	--
	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ		
63	57.1	63.4	63.7	58.3	58.2	54.5	63.9	63.8	60	95
	59.4	62.2	64.5	59.3	58.7	53.2	60.0	64.6		
125	117	121	127	115	116	114	121	127	121	97
	118	125	132	116	117	119	122	127		
250	239	251	265	241	235	257	250	251	247	99
	237	249	262	230	238	248	248	250		
500	490	498	544	474	507	497	503	525	502	100
	484	498	537	482	516	477	507	500		
1000	973	1004	1055	956	999	973	1014	964	995	100
	980	1007	1067	946	1002	960	1022	991		
2000	1893	1879	2014	1774	1894	1805	1841	1719	1876	94
	1955	1908	2078	1835	1962	1836	1885	1734		

[1]The limit of quantitation (LOQ) was 20.0 mg a.i./L.

Table 2

Temperature of Water in the Test Chambers[1]

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Temperature (°C)										
	Day 0	Day 2		Day 5		Day 7		Day 9		Day 12	
	New	Old	New	Old	New	Old	New	Old	New	Old	New
Negative Control	20.4	20.9	21.0	21.0	19.4	20.8	20.4	20.6	19.9	19.5	19.8
60	19.9	20.9	20.5	21.0	19.0	20.7	20.0	20.4	19.7	19.3	19.6
121	19.8	20.8	20.8	20.8	19.0	20.8	19.7	20.6	19.9	19.5	19.6
247	19.9	21.0	20.9	21.0	19.0	20.9	19.8	20.8	19.4	19.4	20.0
502	19.9	20.9	20.9	21.3	19.0	20.9	19.8	20.5	20.8	19.3	20.0
995	20.0	21.0	21.0	20.9	19.0	21.0	19.9	20.4	20.8	20.8	20.1
1876	20.2	21.0	21.0	21.4	19.2	21.0	20.1	20.6	20.8	19.6	20.1

[1]Temperature measured continuously during the test ranged from 18.0°C to 21.0°C.

Table 2 (Continued)

Temperature of Water in the Test Chambers[1]

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Temperature (°C)						
	Day 14		Day 16		Day 19		Day 21
	Old	New	Old	New	Old	New	Old
Negative Control	20.9	20.9	20.5	19.8	20.7	20.3	21.0
60	20.3	20.5	20.4	19.4	21.0	19.7	20.8
121	20.8	20.2	19.9	19.3	21.0	19.5	19.9
247	20.9	20.0	19.6	19.3	20.9	19.5	21.0
502	20.8	20.1	20.7	19.7	20.5	19.6	20.8
995	21.3	20.1	19.8	19.7	21.0	19.7	21.0
1876	21.1	20.3	20.6	19.6	21.0	19.9	21.0

[1]Temperature measured continuously during the test ranged from 18.0°C to 21.0°C.

Table 3

Dissolved Oxygen Content of Water in the Test Chambers[1]

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Dissolved Oxygen Concentration (mg/L)										
	Day 0	Day 1	Day 2		Day 3	Day 4	Day 5		Day 6	Day 7	
	New	Old	Old	New	Old	Old	Old	New	Old	Old	New
Negative Control	7.9	8.2	8.6	8.4	8.6	8.4	8.4	8.6	8.5	8.2	8.1
60	8.2	8.2	8.6	8.5	8.6	8.4	8.4	8.6	8.5	8.2	8.3
121	8.0	8.2	8.6	8.5	8.6	8.4	8.4	8.6	8.5	8.2	8.3
247	8.0	8.2	8.6	8.5	8.6	8.4	8.4	8.6	8.5	8.2	8.2
502	7.9	8.2	8.6	8.6	8.6	8.4	8.4	8.6	8.5	8.2	8.2
995	8.1	8.2	8.6	8.4	8.6	8.4	8.4	8.6	8.5	8.2	8.5
1876	8.2	8.2	8.6	8.5	8.6	8.4	8.4	8.6	8.6	8.2	8.8

[1]A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in freshwater

Table 3 (Continued)

Dissolved Oxygen Content of Water in the Test Chambers[1]

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Dissolved Oxygen Concentration (mg/L)										
	Day 9		Day 12		Day 14		Day 16		Day 19		Day 21
	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Negative Control	8.3	8.4	8.7	8.0	8.2	8.6	8.3	8.4	8.4	8.1	8.3
60	8.3	8.5	8.6	8.7	8.3	8.4	8.2	8.6	8.2	8.1	8.2
121	8.2	8.6	8.6	8.6	8.2	8.4	8.2	8.7	8.4	8.1	8.3
247	8.2	8.6	8.6	8.6	8.2	8.4	8.4	8.6	8.2	8.2	8.0
502	8.3	8.6	8.6	8.6	8.3	8.4	8.2	8.7	8.4	8.4	8.2
995	8.4	8.8	8.6	8.8	8.2	8.4	8.4	8.9	8.5	8.6	8.1
1876	8.3	9.0	8.8	9.0	8.4	9.0	8.3	9.0	8.5	9.0	8.4

[1]A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in freshwater.

Table 4

pH of Water in the Test Chambers

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	pH										
	Day 0	Day 2		Day 5		Day 7		Day 9		Day 12	
	New	Old	New	Old	New	Old	New	Old	New	Old	New
Negative Control	7.9	8.5	8.0	8.5	8.1	8.4	8.2	8.4	8.0	8.5	7.9
60	8.0	8.5	8.2	8.6	8.1	8.4	8.2	8.5	8.1	8.5	8.0
121	8.0	8.6	8.2	8.6	8.1	8.4	8.2	8.5	8.1	8.5	8.0
247	8.0	8.6	8.2	8.6	8.1	8.4	8.3	8.5	8.1	8.5	8.0
502	8.1	8.6	8.3	8.6	8.1	8.5	8.3	8.5	8.1	8.6	8.1
995	8.1	8.6	8.3	8.7	8.2	8.5	8.4	8.5	8.1	8.6	8.1
1876	8.2	8.7	8.4	8.7	8.2	8.6	8.5	8.5	8.2	8.7	8.3

Table 4 (Continued)

pH of Water in the Test Chambers

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	pH						
	Day 14		Day 16		Day 19		Day 21
	Old	New	Old	New	Old	New	Old
Negative Control	8.3	8.2	8.3	8.0	8.3	8.0	8.5
60	8.3	8.1	8.3	8.1	8.3	8.0	8.5
121	8.4	8.1	8.4	8.1	8.3	8.0	8.6
247	8.4	8.2	8.4	8.1	8.4	8.0	8.5
502	8.5	8.2	8.5	8.2	8.4	8.1	8.6
995	8.5	8.2	8.5	8.3	8.5	8.2	8.6
1876	8.6	8.2	8.6	8.4	8.6	8.4	8.6

Table 5

Specific Conductance, Hardness and Alkalinity in the Negative Control and Highest Treatment Group

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Negative Control

	Time			
Parameter	Day 0	Day 7	Day 14	Day 21
Specific Conductance (µmhos/cm)	325	335	340	330
Hardness (mg/L as $CaCO_3$)	128	128	132	128
Alkalinity (mg/L as $CaCO_3$)	178	180	182	184

1876 mg a.i./L

	Time			
Parameter	Day 0	Day 7	Day 14	Day 21
Specific Conductance (µmhos/cm)	800	800	800	850
Hardness (mg/L as $CaCO_3$)	140	140	128	132
Alkalinity (mg/L as $CaCO_3$)	178	180	176	184

Table 6

Summary of Cumulative Percent Mortality and Treatment-Related Effects[1] for First-Generation Daphnids

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Mean Measured Test Concentration (mg a.i./L)	Cumulative Observations to Day 7			Cumulative Observations to Day 14			Cumulative Observations to Day 21		
	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects
Negative Control	0	0	10 AN	0	0	10 AN	10	0	8AN, 1D
60	0	0	10 AN	0	0	10 AN	0	0	10 AN
121	0	0	10 AN	0	0	10 AN	0	0	10 AN
247	0	0	10 AN	0	0	10 AN	0	0	10 AN
502	0	0	10 AN	0	0	10 AN	0	0	10 AN
995	0	0	6AN, 4D	30	0	5AN, 1D, 1D,G	30	0	6AN, 1D
1876	20	0	8 G	80	0	2 G	80*	0	2 D,G

[1]Observed Effects: AN = Appears Normal, D = Discoloration, G = Appears Smaller

*Indicates a significant difference from the negative control on Day 21 using Fisher's exact test ($p \leq 0.05$).

Table 7

Summary of *Daphnia magna* Reproduction During the Life-Cycle Toxicity Test

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Mean Measured Test Concentration (mg a.i./L)	Number of Surviving Daphnids	Mean Live Young/ Surviving Adult Daphnid (±SD)	First Day of Reproduction	Total Number of Dead/Immobile Neonates	Total Number of Aborted Eggs
Negative Control	9	86 (±27)	8	0	0
60	10	173 (±34)	8	0	0
121	10	149 (±47)	8	0	0
247	10	167 (±30)	8	0	0
502	10	89 (±26)	8	0	0
995	7	43* (±28)	8	0	0
1876[1]	2	14 (±14)	11	3	0

[1]This treatment was excluded from the statistical analyses of reproduction due to a statistically significant survival effect.

*Indicates a statistically significant difference from the control using the Bonferroni t-test ($p \leq 0.05$).

Table 8

Summary of Length and Dry Weight of Surviving
First-Generation Daphnids

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Mean Measured Test Concentration (mg a.i./L)	Number of Surviving Daphnids	Total Length (mm) Mean (±SD)	Dry Weight (mg) Mean (±SD)
Negative Control	9	4.18 (±0.182)	0.80 (±0.29)
60	10	4.89 (±0.111)	1.22 (±0.31)
121	10	4.78 (±0.207)	1.21 (±0.34)
247	10	4.77 (±0.206)	0.96 (±0.16)
502	10	4.26 (±0.480)	0.88 (±0.16)
995	7	3.69* (±0.352)	0.51 (±0.16)
1876[1]	2	2.68 (±0.106)	0.10 (±0.0)

[1]This treatment was excluded from the statistical analyses of growth due to a statistically significant survival effect.
*Indicates a significant difference from the negative control using Wilcoxon's rank sum test ($p \leq 0.05$).

Table 9

Cumulative Percent Mortality/Immobility and Treatment-Related Effects[1] for Second-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Concentration (mg a.i./L)	Replicate	Daphnia/ Replicate	2 Hours			24 Hours			Percent Immobile and Dead	48 Hours			Percent Immobile and Dead
			Number Dead	Number Immobile	Effects	Number Dead	Number Immobile	Effects		Number Dead	Number Immobile	Effects	
Negative Control	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
60	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
121	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
247	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
502	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
995	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	

[1] Observed Effects: AN = Appears Normal

Appendix 1

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured During the 4-Week Period Immediately Preceding the Test

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

	Mean	Range
Specific Conductance (μmhos/cm)	309 (N = 4)	305 – 310
Hardness (mg/L as $CaC0_3$)	131 (N = 4)	124 - 136
Alkalinity (mg/L as $CaC0_3$)	178 (N = 4)	176 - 182
pH	8.0 (N = 4)	8.0 – 8.1

Appendix 2
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Well Water[1]

Component	Measured Concentration	Component	Measured Concentration
Pesticides and Organics			
Aclonifen	<0.03 µg/L	Dimethomorf	<0.05 µg/L
Alachlor	<0.01 µg/L	Disulfoton	<0.02 µg/L
Ametryn	<0.01 µg/L	DMST	<0.05 µg/L
Atrazin	<0.01 µg/L	Dodemorf	<0.01 µg/L
Azinphos-ethyl	<0.04 µg/L	Endosulfan-α	<0.01 µg/L
Azinphos-methyl	<0.08 µg/L	Endosulfan-β	<0.01 µg/L
Azoxystrobin	<0.25 µg/L	Endosulfan-sulfaat	<0.02 µg/L
Bifenthrin	<0.05 µg/L	Epoxiconazole	<0.05 µg/L
Bioallethrin	<0.05 µg/L	Eptam	<0.02 µg/L
Bitertanol	<0.05 µg/L	Esfenvaleraat	<0.02 µg/L
Bromacil	<0.05 µg/L	Ethion	<0.05 µg/L
Bromophos	<0.02 µg/L	Ethofumesaat	<0.02 µg/L
Bromophos-ethyl	<0.02 µg/L	Ethoprophos	<0.01 µg/L
Broompropylaat	<0.02 µg/L	Etridiazole	<0.02 µg/L
Bupirimaat	<0.05 µg/L	Etrimphos	<0.05 µg/L
Carbaryl	<0.05 µg/L	Fenarimol	<0.05 µg/L
Carbofuran	<0.03 µg/L	Fenchlorphos	<0.01 µg/L
Carboxin	<0.02 µg/L	Fenitrothion	<0.03 µg/L
Chlorfenvinphos	<0.02 µg/L	Fenoxycarb	<0.03 µg/L
Chloridazon	<0.05 µg/L	Fenpiclonil	<0.05 µg/L
Chlorpropham	<0.02 µg/L	Fenpropathrin	<0.25 µg/L
Chlorpyriphos	<0.01 µg/L	Fenpropimorf	<0.01 µg/L
Chlorpyriphos-methyl	<0.01 µg/L	Fenthion	<0.01 µg/L
Chlorthalonil	<0.04 µg/L	Fenvaleraat	<0.02 µg/L
Coumaphos	<0.02 µg/L	Fluazifop-butyl	<0.02 µg/L
Cyanazin	<0.05 µg/L	Fluoroglycofen-ethyl	<0.02 µg/L
Cyfluthrin	<0.05 µg/L	Fluroxypyr-meptyl	<0.05 µg/L
Cypermethrin	<0.25 µg/L	Flutolanil	<0.02 µg/L
Cyproconazole	<0.05 µg/L	Fonophos	<0.01 µg/L
Deltamethrin	<0.02 µg/L	Furalaxyl	<0.02 µg/L
Demeton	<0.02 µg/L	Heptenophos	<0.02 µg/L
Demeton-o	<0.02 µg/L	Imazalil	<0.01 µg/L
Desethylatrazin	<0.01 µg/L	Iprodion	<0.05 µg/L
Desisopropylatrazin	<0.02 µg/L	Kresoxim-methyl	<0.02 µg/L
Desmetryn	<0.01 µg/L	Lenacil	<0.05 µg/L
Diazinon	<0.01 µg/L	Lindane	<0.02 µg/L
Dichlobenil	<0.01 µg/L	Malathion	<0.02 µg/L
Dichloran	<0.03 µg/L	Metalaxyl	<0.05 µg/L
Dichlorbenzamide	<0.02 µg/L	Metamitron	<0.05 µg/L
Dichlorfenthion	<0.01 µg/L	Metazachlor	<0.02 µg/L
Dichlorfluanid	<0.03 µg/L	Methidathion	<0.02 µg/L

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Appendix 2 (Continued)
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Well Water[1]

Pesticides and Organics (Page 2)			
Component	Measured Concentration	Component	Measured Concentration
Dichlorvos	<0.01 µg/L	Methoxychlor	<0.01 µg/L
Dicofol	<0.25 µg/L	Metolachlor	<0.01 µg/L
Diethyltoluamide	<0.02 µg/L	Metribuzin	<0.02 µg/L
Difenoconazole	<0.03 µg/L	Mevinphos	<0.01 µg/L
Dimethoate	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazin	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalon	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbutylazin	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftaalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclophos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazin	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazool	<0.05 µg/L	Triallaat	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinchlozolin	<0.01 µg/L
Metals			
Magnesium	11.0 mg/L	Nickel	<1.1 µg/L
Sodium	18.0 mg/L	Copper	<0.7 µg/L
Calcium	29 mg/L	Zinc	<0.25 µg/L
Iron	<0.015 mg/L	Molybdenum	<0.3 µg/L
Potassium	1.1 mg/L	Silver	<0.2 µg/L
Aluminum	<0.02 mg/L	Cadmium	<0.1 µg/L
Manganese	<0.1 µg/L	Arsenic	<0.5 µg/L
Beryllium	<0.2 µg/L	Mercury	<0.025 µg/L
Chromium	<0.5 µg/L	Selenium	<0.5 µg/L
Cobalt	<0.2 µg/L		

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Appendix 3

THE ANALYSIS OF PFBS IN FRESHWATER

IN SUPPORT OF

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-130

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFBS: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-130

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

PRINCIPAL INVESTIGATOR:

Raymond J. Van Hoven, Ph.D.
Scientist

4/11/01
DATE

MANAGEMENT:

Willard B. Nixon, Ph.D.
Director, Analytical Chemistry

4/11/01
DATE

Introduction

Freshwater samples were collected from a semi-static life-cycle aquatic toxicity study designed to determine the effects of PFBS (Perfluorobutane Sulfonate, Potassium Salt) to the cladoceran (*Daphnia magna*). This study was conducted by Wildlife International, Ltd. and identified as Project Number 454A –130. The analyses of these water samples were performed at Wildlife International, Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were received for analysis between February 7, 2001 and February 28, 2001 and were prepared for analysis on each sample receipt day.

Analytical Standard

The analytical standard was received from 3M Environmental Technology and Safety Services on March 27, 2000, assigned Wildlife International, Ltd. Identification number 5216, and stored under ambient conditions. The analytical standard, a white powder, was identified as: Potassium Perfluorobutane Sulfonate , Lot 2), expiration date: March 2010. The analytical standard was further identified with the 3M Environmental Laboratory test control and reference number TCR .

The test substance had a reported purity of 97.90%. A subsequent revision of the certificate of analysis indicated a purity of 97.3% and an Expiration/Reassessment Date of January 17, 2002. The analytical standard was the same material and lot number as the test substance (Wildlife International, Ltd. Identification number 5292). The analytical standard was used to prepare calibration standards and matrix fortification samples.

Analytical Method

Water samples were analyzed according to the method entitled "Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Freshwater" (Wildlife International, Ltd. Project No. 454C-115). Samples were diluted in a 50% methanol : 50% NANOpure® water solution so that they fell within the calibration range of the PFBS methodology. Aliquots of the dilutions were transferred to autosampler vials and submitted for analysis by direct injection. Concentrations of PFBS in freshwater samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) interfaced with a Perkin-

Elmer API 100LC mass spectrometer (single quadrupole) operated in selective ion monitoring (SIM) detection mode. The mass spectrometer was equipped with a Perkin-Elmer TurboIonSpray ion source.

Chromatographic separations were achieved using a Keystone PRISM RP column (30 mm × 1.5 mm, 3 µm particle size) fitted with a Keystone Javelin C_{18} Guard Cartridge (20 mm × 2 mm). The instrument parameters are summarized in Table 1 and a method flowchart is provided in Figure 1.

Primary and Secondary Stock Solutions

All primary and secondary stock preparations were adjusted for the purity of the analytical standard (97.90%). A 10.0 mg a.i./mL primary stock solution of PFBS in methanol was prepared by weighing 1.0243 g of the analytical standard and bringing to final volume with methanol. Secondary stock solutions (1000, 100, 10.0, 1.00, and 0.100 mg a.i./L) of PFBS in methanol were prepared by serial volumetric dilution from the primary stock.

Calibration Standards and Calibration Curves

Calibration standards were prepared in 50:50 methanol: NANOpure® water by appropriate dilutions of the 10.0 mg a.i./L stock solution of PFBS in methanol. The calibration standards of PFBS, ranging in concentration from 0.0100 to 0.0500 mg a.i./L, were analyzed with each sample set. Five calibration standards (different concentrations) were analyzed with the samples. The calibration standard series was injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve is presented in Figure 2. The concentration of PFBS in the samples was determined by substituting the peak area responses into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

Limit of Quantitation

The method limit of quantitation (LOQ) for these analyses was set at 20.0 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (2000).

Matrix Blank and Fortification Samples

Eight matrix blank samples were analyzed to determine possible interference. No interferences were observed at or above the LOQ during samples analyses (Table 2). A representative ion chromatogram of a matrix blank is presented in Figure 5.

Freshwater was either fortified with the appropriate PFBS stock solution in methanol (30.0 mg a.i./L), or directly fortified (*i.e.* without use of carrier solvent) with PFBS (400 and 2500 mg a.i./L). Direct fortifications were required for the higher fortification levels due to the limitation in preparing a sufficiently concentrated methanol stock of PFBS. The fortified samples were analyzed concurrently with the test samples to determine the mean procedural recovery (Table 3). Sample concentrations were not corrected for the mean overall procedural recovery of 96.3%. A representative ion chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-130-9, nominal concentration of 500 mg a.i./L in freshwater.

First Initial Volume: 0.100 mL
First Final Volume: 10.0 mL
Second Initial Volume: 0.100 mL
Second Final Volume: 25.0 mL
Dilution Factor: 25000
PFBS Peak Area: 221526

Calibration curve equation:
Slope: 10184392
Intercept: 21932.54
Curve regression weighted 1/x

$$\text{PFBS (mg a.i./L) measured at instrument} = \frac{\text{peak area - (y-intercept)}}{\text{slope}}$$

PFBS (mg a.i./L) in sample = PFBS measured at instrument (mg a.i./L) × dilution factor

$$= \frac{221526 - 21932.54}{10184392} \times 25000$$

$$= 489.9$$

$$\text{Percent of Nominal Concentration} = \frac{\text{PFBS (mg a.i./L) in sample}}{\text{PFBS (mg a.i./L) nominal}} \times 100$$

$$= \frac{489.9}{500} \times 100 = 98.0\%$$

Calculated with HPLC/MS instrument software: MacQuan, version 1.6.

RESULTS

Sample Analysis

Freshwater samples were collected from a semi-static life-cycle toxicity study with the cladoceran (*Daphnia magna*) at test initiation, February 7, 2001 (Day 0), on February 9, 2001 (Day 2 - new solutions), on February 12, 2001 (Day 5 - old solutions), on February 16, 2001 (Day 9 – new solutions), on February 19, 2001 (Day 12 – old solutions), on February 23, 2001 (Day 16 – new solutions), on February 26, 2001 (Day 19 – old solutions) and at test termination on February 28, 2001 (Day 21 - old solutions). The measured concentrations of PFBS in the samples collected at initiation of exposure of the test organisms (Hour 0) ranged from 90.7 to 98.0% of the nominal concentrations (Table 4). Samples collected at Day 2, Day 5, Day 9, Day 12, Day 16 and Day 19 had measured concentration ranges of 94.0 to 101%, 101 to 109%, 88.7 to 96.5%, 92.4 to 103%, 84.5 to 103% and 92.0 to 102% of nominal values, respectively. Samples collected at test termination (Day 21) had a measured concentration range of 85.9 to 105% of nominal values. A representative ion chromatogram of a test sample is shown in Figure 7.

Table 1

Typical HPLC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 100LC Mass Spectrometer operated in Selective Ion Monitoring (SIM) Mode
ION SOURCE:	Perkin-Elmer TurboIonSpray
ANALYTICAL COLUMN:	Keystone PRISM RP (30 mm × 1.5 mm, 3-µm particle size)
GUARD COLUMN:	Keystone Javelin C_{18} cartridge (20 mm × 2 mm)
OVEN TEMPERATURE:	40°C
STOP TIME:	3.00 min
FLOW RATE:	200 µL/min
MOBILE PHASE:	25% NANOpure® Water with 0.1% Ammonium Formate: 75% Methanol
INJECTION VOLUME:	5.0 µL
PFBS PEAK RETENTION TIME:	Approximately 2.1 minutes
PFBS MONITORED MASS:	299.0 amu

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of PFBS (mg a.i./L) [2]
Number (454A-130-)	Preparation Date (Day) [1]	
MAB-1	0	< LOQ
MAB-2	2	< LOQ
MAB-3	5	< LOQ
MAB-4	9	< LOQ
MAB-5	12	< LOQ
MAB-6	16	< LOQ
MAB-7	19	< LOQ
MAB-8	21	< LOQ

[1] Preparation Date refers to the Day during the definitive test that these quality control samples were prepared concurrent with sampling of the test samples.

[2] The limit of quantitation (LOQ) was 20.0 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.0100 mg/L) and the dilution factor of the matrix blank samples (2000).

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454A-130-)	Preparation Date (Day)[1]	Concentrations of PFBS (mg a.i./L)		Percent of Nominal[2]
		Fortified	Measured[2]	
MAS-1	0	30.0	31.6	105
MAS-4	2	30.0	31.0	104
MAS-7	5	30.0	29.8	99.3
MAS-10	9	30.0	30.6	102
MAS-13	12	30.0	30.0	100
MAS-16	16	30.0	29.5	98.4
MAS-19	19	30.0	31.2	104
MAS-22	21	30.0	31.1	104
MAS-2	0	400	390	97.5
MAS-5	2	400	376	94.0
MAS-8	5	400	348	87.1
MAS-11	9	400	361	90.3
MAS-14	12	400	350	87.6
MAS-17	16	400	361	90.2
MAS-20	19	400	372	93.0
MAS-23	21	400	414	103
MAS-3	0	2500	2355	94.2
MAS-6	2	2500	2346	93.8
MAS-9	5	2500	2275	91.0
MAS-12	9	2500	2346	93.8
MAS-15	12	2500	2212	88.5
MAS-18	16	2500	2409 [3]	96.4
MAS-21	19	2500	2356	94.2
MAS-24	21	2500	2481	99.2
			Mean =	96.3
			Standard Deviation =	5.63
			CV =	5.84%
			N =	24

[1] Preparation Date refers to the Day during the definitive test that these quality control samples were prepared concurrent with sampling of the test samples.

[2] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

[3] Mean of duplicate redilutions (2399 and 2419 mg a.i./L) of original fortification. A dilution error occurred during original sample preparation.

Table 4

Measured Concentrations of PFBS in Freshwater Samples from a Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-130-)	Sampling Time (Day)	PFBS Measured Concentration[1] (mg a.i./L)	Percent of Nominal[1]
0.0	1	0	<LOQ[2]	--
	2	0	<LOQ	--
	15	2	<LOQ	--
	16	2	<LOQ	--
	29	5	<LOQ	--
	30	5	<LOQ	--
	43	9	<LOQ	--
	44	9	<LOQ	--
	57	12	<LOQ	--
	58	12	<LOQ	--
	71	16	<LOQ	--
	72	16	<LOQ	--
	85	19	<LOQ	--
	86	19	<LOQ	--
	99	21	<LOQ	--
	100	21	<LOQ	--
63	3	0	57.1	90.7
	4	0	59.4	94.3
	17	2	63.4	101
	18	2	62.2	98.7
	31	5	63.7	101
	32	5	64.5	102
	45	9	58.3	92.6
	46	9	59.3	94.1
	59	12	58.2	92.4
	60	12	58.7	93.2
	73	16	54.5	86.5
	74	16	53.2	84.5
	87	19	63.9	101
	88	19	60.0	95.2
	101	21	63.8	101
	102	21	64.6	103

[1] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

[2] The limit of quantitation (LOQ) was 20.0 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (2000).

Table 4 (Continued)

Measured Concentrations of PFBS in Freshwater Samples from a Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-130-)	Sampling Time (Day)	PFBS Measured Concentration[1] (mg a.i./L)	Percent of Nominal[1]
125	5	0	117	93.3
	6	0	118	94.8
	19	2	121	96.7
	20	2	125	99.9
	33	5	127	102
	34	5	132	105
	47	9	115	92.4
	48	9	116	93.0
	61	12	116	92.5
	62	12	117	93.7
	75	16	114	91.2
	76	16	119	95.5
	89	19	121	96.6
	90	19	122	97.7
	103	21	127	102
	104	21	127	101
250	7	0	239	95.7
	8	0	237	94.8
	21	2	251	101
	22	2	249	99.7
	35	5	265	106
	36	5	262	105
	49	9	241	96.5
	50	9	230	92.1
	63	12	235	94.1
	64	12	238	95.0
	77	16	257	103
	78	16	248	99.1
	91	19	250	100
	92	19	248	99.3
	105	21	251	100
	106	21	250	100

[1] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

Table 4 (Continued)

Measured Concentrations of PFBS in Freshwater Samples from a Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-130-)	Sampling Time (Day)	PFBS Measured Concentration[1] (mg a.i./L)	Percent of Nominal[1]
500	9	0	490	98.0
	10	0	484	96.7
	23	2	498	99.5
	24	2	498	99.7
	37	5	544	109
	38	5	537	107
	51	9	474	94.9
	52	9	482	96.4
	65	12	507	101
	66	12	516	103
	79	16	497	99.5
	80	16	477	95.4
	93	19	503	101
	94	19	507	101
	107	21	525	105
	108	21	500	100
1000	11	0	973	97.3
	12	0	980	98.0
	25	2	1004	100
	26	2	1007	101
	39	5	1055	106
	40	5	1067	107
	53	9	956	95.6
	54	9	946	94.6
	67	12	999	99.9
	68	12	1002	100
	81	16	973	97.3
	82	16	960	96.0
	95	19	1014	101
	96	19	1022	102
	109	21	964	96.4
	110	21	991	99.1

[1] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

Table 4 (Continued)

Measured Concentrations of PFBS in Freshwater Samples from a Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-130-)	Sampling Time (Day)	PFBS Measured Concentration[1] (mg a.i./L)	Percent of Nominal[1]
2000	13	0	1893	94.7
	14	0	1955	97.8
	27	2	1879	94.0
	28	2	1908	95.4
	41	5	2014	101
	42	5	2078	104
	55	9	1774	88.7
	56	9	1835	91.8
	69	12	1894	94.7
	70	12	1962	98.1
	83	16	1805	90.2
	84	16	1836	91.8
	97	19	1841	92.0
	98	19	1885	94.2
	111	21	1719	85.9
	112	21	1734	86.7

[1] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

METHOD OUTLINE FOR THE ANALYSIS OF PFBS IN FRESHWATER

Prepare matrix fortification samples in freshwater as follows: For target PFBS concentrations ≤ 200 mg a.i./L, fortify freshwater with the appropriate stock solution of PFBS. For target PFBS concentrations > 200 mg a.i./L, prepare by weighing the requisite amount of PFBS test substance on an analytical balance and transferring directly into a Class A volumetric flask partially filled with freshwater. Rinse weighing paper and the sides of the flask with repeat freshwater rinses. Swirl the flask to dissolve the test substance and then bring to final volume with freshwater. Sonicate as appropriate and mix with several repeat inversions. The matrix blank is unfortified freshwater.

↓

Prepare appropriate dilutions of study and QC samples to within the calibration range of the PFBS LCMS methodology: Partially fill Class A volumetric flasks with 50% methanol : 50% NANOpure® water dilution solvent. Add the appropriate volume of sample and bring to volume with dilution solvent. Perform secondary dilutions as necessary. Process matrix blank samples using the same dilution and aliquot volume as for the lowest fortification level. Mix well by several repeat inversions.

↓

Ampulate samples and submit for LCMS analysis.

Figure 1. Analytical method flowchart for the analysis of PFBS in freshwater.

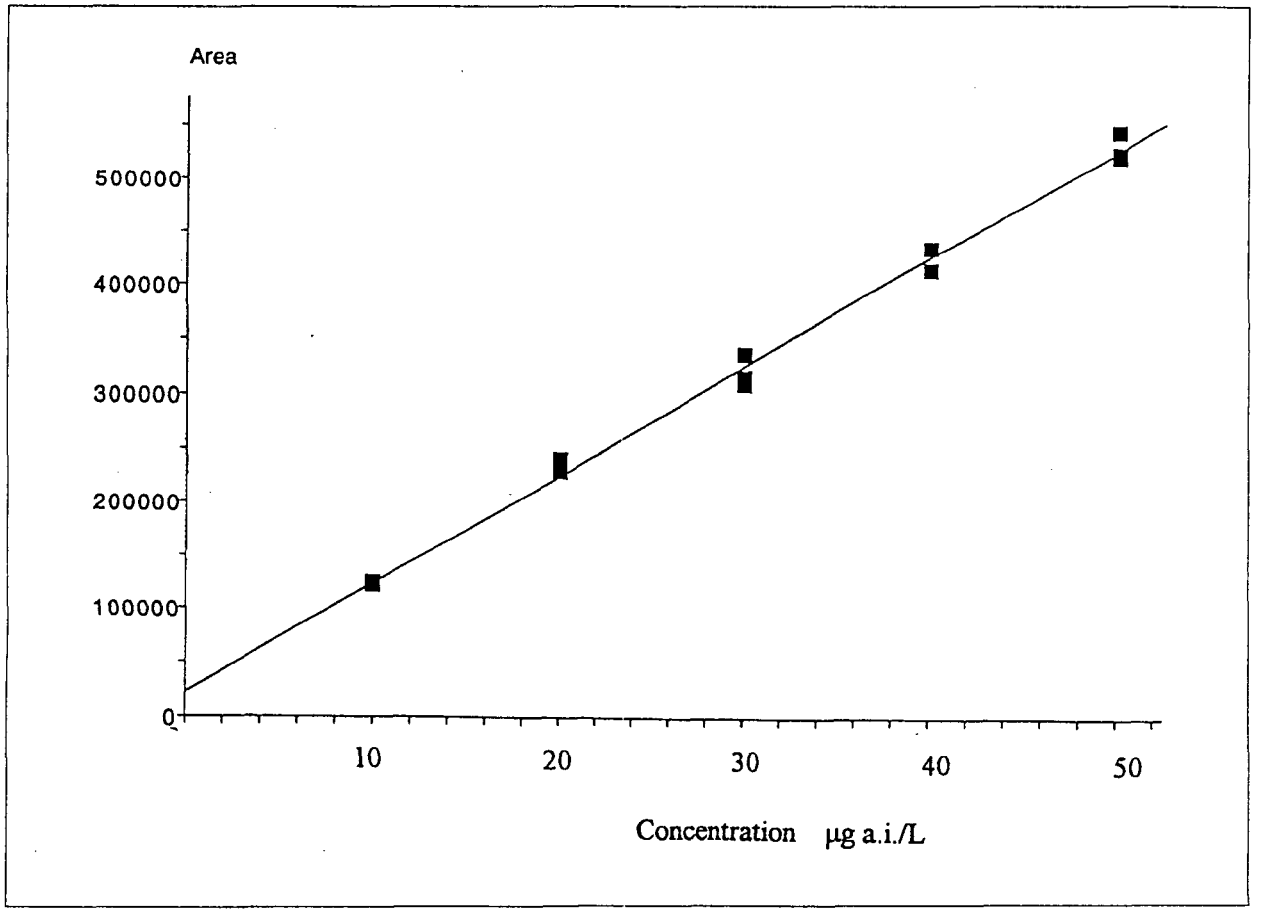

Figure 2. A typical calibration curve for PFBS. Slope = 10184392; Intercept = 21932.54; r = 0.99818.

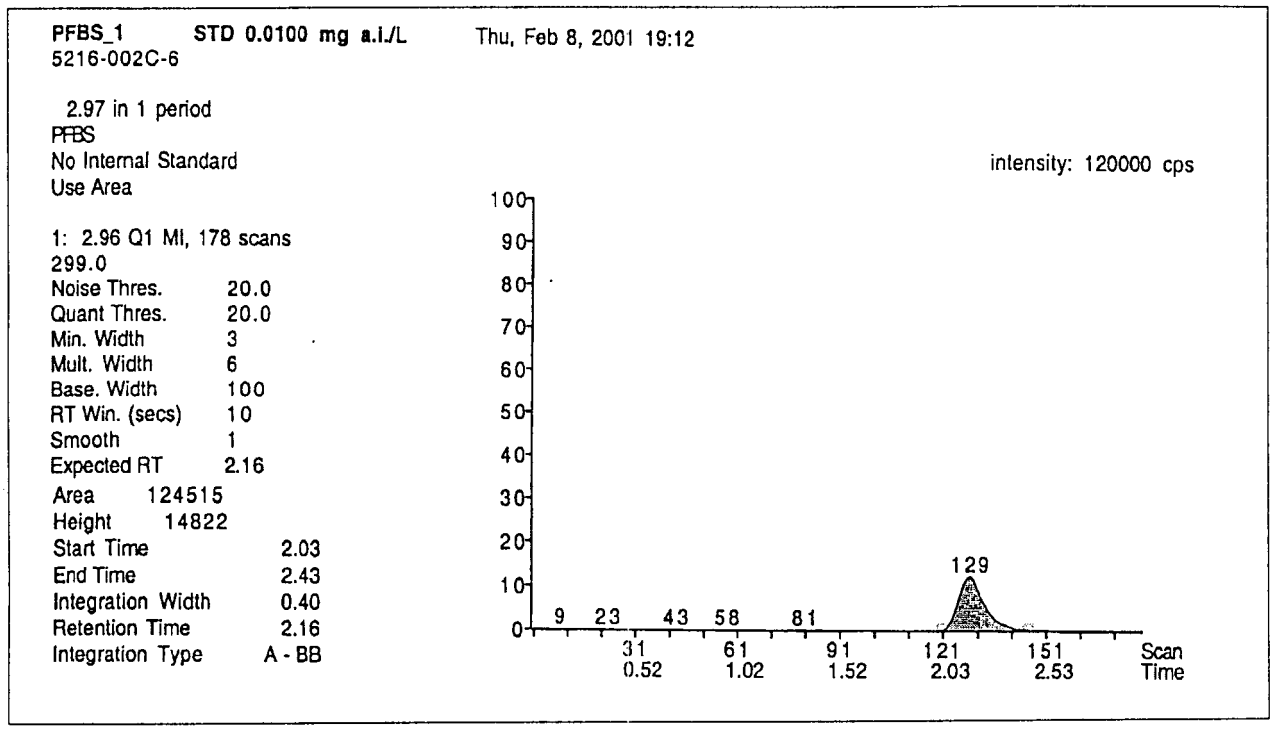

Figure 3. A representative ion chromatogram of a low-level (0.0100 mg a.i./L) PFBS standard.

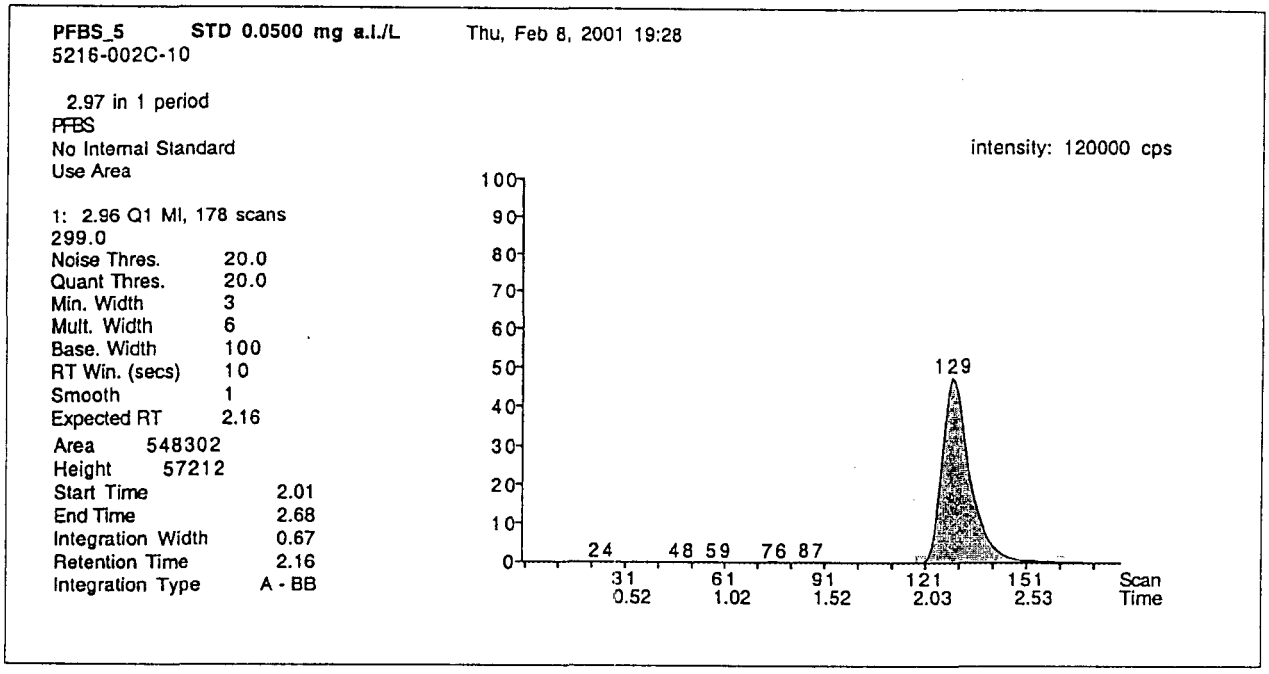

Figure 4. A representative ion chromatogram of a high-level (0.0500 mg a.i./L) PFBS standard.

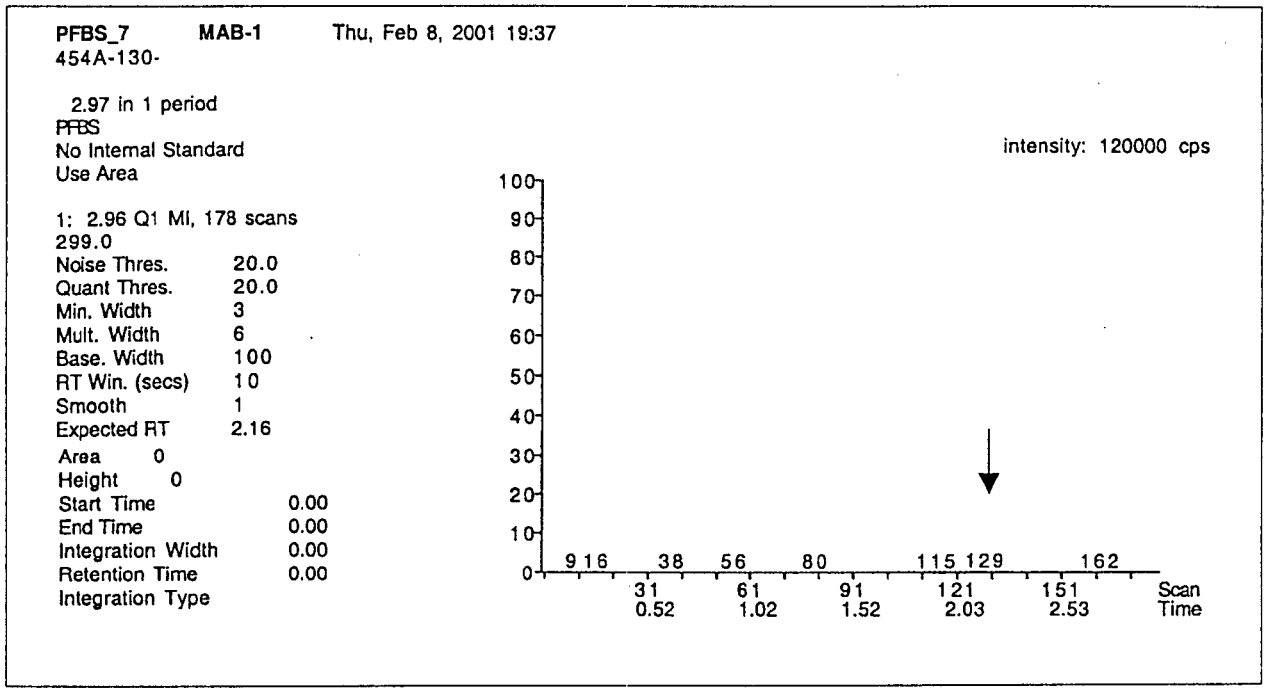

Figure 5. A representative ion chromatogram of a matrix blank sample (454A-130-MAB-1). The arrow indicates the retention time of PFBS.

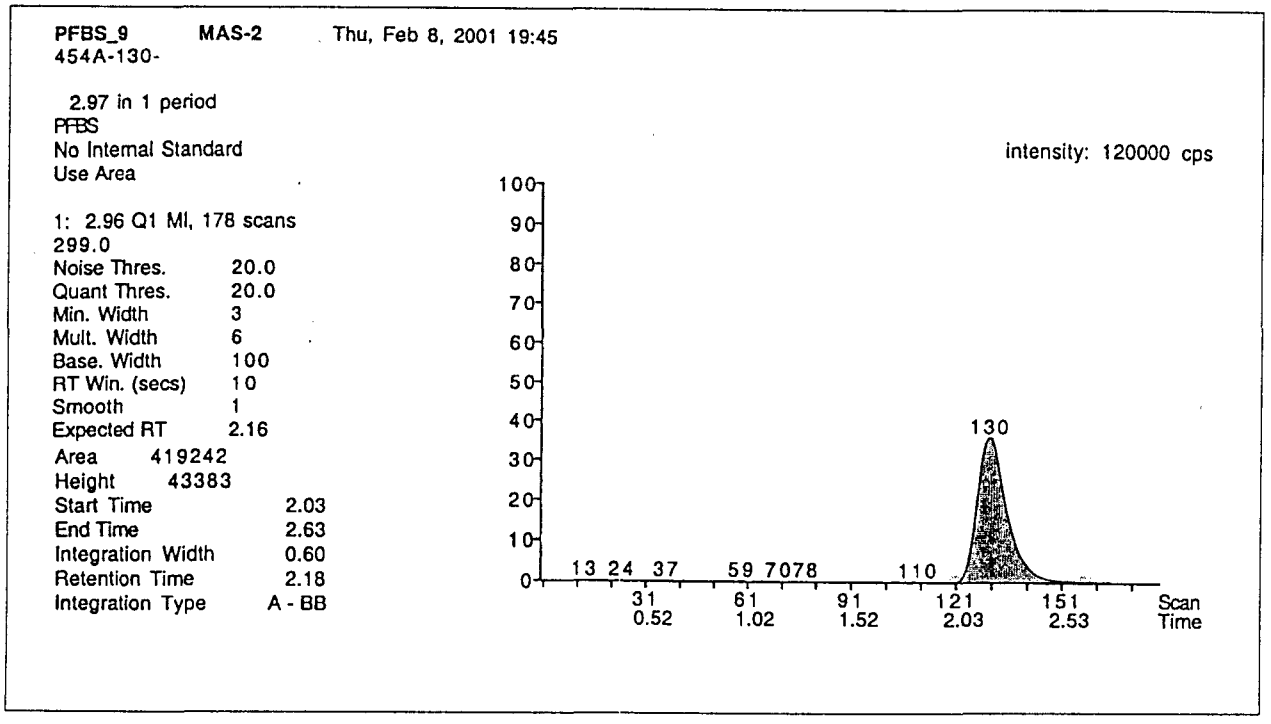

Figure 6. A representative ion chromatogram of a matrix fortification sample (454A-130-MAS-2, nominal PFBS concentration of 400 mg a.i./L, dilution factor = 10000x).

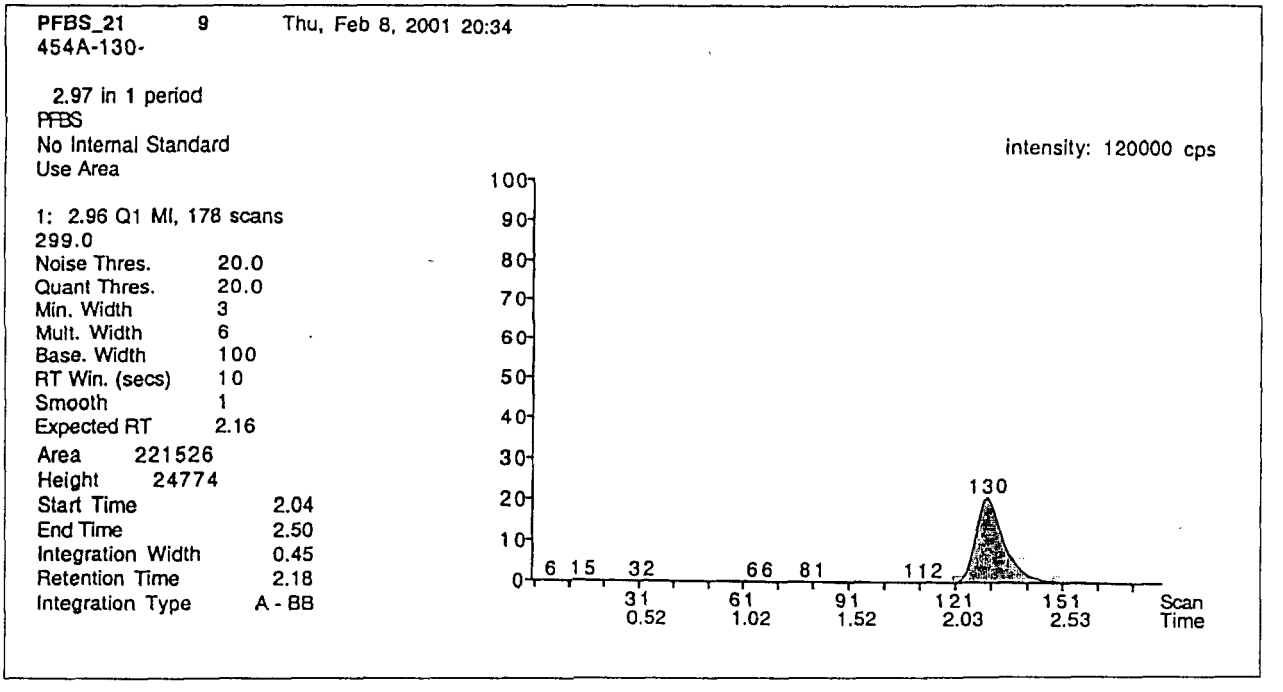

Figure 7. A representative ion chromatogram of a test sample (454A-130-9, nominal PFBS concentration of 500 mg a.i./L, dilution factor = 25000x).

Appendix 4

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 0			Day 1			Day 2			Day 3		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 4			Day 5			Day 6		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 7			Day 8			Day 9		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 10			Day 11			Day 12		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 13			Day 14			Day 15		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 16			Day 17			Day 18		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 19			Day 20			Day 21		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
60	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
121	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 0			Day 1			Day 2			Day 3		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
502	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
995	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 4			Day 5			Day 6		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
502	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
995	A	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 7			Day 8			Day 9		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
502	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
995	A	1	0	0	1 D	0	0	1 D	0	1	–
	B	1	0	0	1 D	0	0	1 D	0	0	1 D
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 D	0	0	1 D	0	0	1 D
	F	1	0	0	1 D	0	0	1 D	0	0	1 D
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	1	–	0	1	–

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 10			Day 11			Day 12		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
502	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
995	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	0	1 D	0	0	1 D	0	0	1 D
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	0	1 D,G	0	0	1 D,G	0	0	1 D,G
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	1	--	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 13			Day 14			Day 15		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
502	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
995	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	0	1 D	0	0	1 D	0	0	1 D
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	0	1 D,G	0	0	1 D,G	0	0	1 D,G
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 D
	J	1	0	1	--	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 16			Day 17			Day 18		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
502	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
995	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	0	1 D	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 D	0	0	1 AN	0	0	1 AN
	J	1	0	1	--	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 19			Day 20			Day 21		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
247	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
502											
	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
995											
	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	0	1 D	0	0	1 D	0	0	1 D
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	1	--	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 0			Day 1			Day 2			Day 3		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	1	--
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	G	1	0	0	1 AN	1	0	--	0	1	--	0	1	--
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	0	0	1 G

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 4			Day 5			Day 6		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	0	1 G	0	0	1 G	0	0	1 G
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	0	1 G	0	0	1 G	0	0	1 G
	D	1	0	0	1 G	0	0	1 G	0	0	1 G
	E	1	0	0	1 G	0	0	1 G	0	0	1 G
	F	1	0	0	1 G	0	0	1 G	0	0	1 G
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	0	1 G	0	0	1 G	0	0	1 G
	I	1	0	0	1 G	0	0	1 G	0	0	1 G
	J	1	0	0	1 G	0	0	1 G	0	0	1 G

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 7			Day 8			Day 9		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	0	1 G	0	0	1 G	0	0	1 G
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	0	1 G	0	0	1 G	0	0	1 G
	D	1	0	0	1 G	0	0	1 G	0	0	1 G
	E	1	0	0	1 G	0	0	1 G	0	0	1 G
	F	1	0	0	1 G	0	0	1 G	0	0	1 G
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	0	1 G	0	0	1 G	0	0	1 G
	I	1	0	0	1 G	0	0	1 G	0	0	1 G
	J	1	0	0	1 G	0	0	1 G	0	0	1 G

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 10			Day 11			Day 12		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	0	1 G	0	0	1 G	0	0	1 G
	D	1	0	0	1 G	0	1	--	0	1	--
	E	1	1	0	1 G	1	0	1 G	0	1	--
	F	1	0	0	1 G	1	0	1 G	0	1	--
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	0	1 G	0	0	1 G	0	0	1 G
	I	1	0	0	1 G	0	0	1 G	0	0	1 G
	J	1	0	0	1 G	0	0	1 G	0	0	1 G

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 13			Day 14			Day 15		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	0	1 G	0	1	--	0	1	--
	D	1	0	1	--	0	1	--	0	1	--
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	0	1 G	0	0	1 G	0	0	1 G
	I	1	0	0	1 G	0	0	1 G	0	0	1 G
	J	1	0	0	1 D,G	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 16			Day 17			Day 18		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	1	--	0	1	--	0	1	--
	D	1	0	1	--	0	1	--	0	1	--
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	0	1 G	0	0	1 G	0	0	1 G
	I	1	0	0	1 G	0	0	1 G	0	0	1 G
	J	1	0	1	--	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 4 (Continued)

Cumulative Percent Mortality and Treatment-Related Effects[1] - First-Generation Daphnids

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed	Day 19			Day 20			Day 21		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
1876	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	1	--	0	1	--	0	1	--
	D	1	0	1	--	0	1	--	0	1	--
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	0	1 D, G	0	0	1 D, G	0	0	1 D, G
	I	1	0	0	1 G	0	0	1 D, G	0	0	1 D, G
	J	1	0	1	--	0	1	--	0	1	--

[1] Observed Effects: AN = Appeared Normal, D = Discoloration (Pale), G = Small

Appendix 5

Neonate Production

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water	Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Day 0	2	5	7	9	12	14	16	19	21	Total
Negative Control	A	0	0	0	0	0	31	31	14	19	0	95
	B	0	0	0	0	9	27	26	--	--	--	62[1]
	C	0	0	0	0	0	0	16	0	6	0	22
	D	0	0	0	0	0	0	22	36	24	0	82
	E	0	0	0	0	0	0	21	27	32	0	80
	F	0	0	0	0	0	7	26	40	17	0	90
	G	0	0	0	0	0	24	44	28	22	0	118
	H	0	0	0	0	0	17	38	30	0	16	101
	I	0	0	0	0	0	33	20	28	0	25	106
	J	0	0	0	0	0	12	35	0	18	15	80
												$\bar{x}$ (± SD) = 86 (± 27)
60	A	0	0	0	0	5	13	57	61	27	0	163
	B	0	0	0	0	4	38	50	62	35	0	189
	C	0	0	0	0	0	18	45	52	28	0	143
	D	0	0	0	0	0	26	44	52	28	0	150
	E	0	0	0	0	3	55	0	47	32	0	137
	F	0	0	0	0	35	57	0	61	54	25	232
	G	0	0	0	0	18	56	0	52	57	30	213
	H	0	0	0	0	2	32	54	44	18	0	150
	I	0	0	0	0	0	31	44	49	25	0	149
	J	0	0	0	0	6	39	15	33	69	45	207
												$\bar{x}$ (± SD) = 173 (± 34)

[1]This value excluded from calculation of the mean due to adult death.

Appendix 5 (Continued)

Neonate Production

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water	Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Day 0	2	5	7	9	12	14	16	19	21	Total
121	A	0	0	0	0	0	15	27	20	0	9	71
	B	0	0	0	0	0	18	46	58	30	0	152
	C	0	0	0	0	0	12	31	30	10	0	83
	D	0	0	0	0	0	74	0	48	51	0	173
	E	0	0	0	0	5	50	0	57	55	27	194
	F	0	0	0	0	4	55	0	43	33	9	144
	G	0	0	0	0	0	74	0	53	34	33	194
	H	0	0	0	0	4	39	57	57	51	0	208
	I	0	0	0	0	0	34	46	45	29	0	154
	J	0	0	0	0	0	16	41	0	43	17	117
												$\bar{x}$ (± SD) = 149 (± 47)
247	A	0	0	0	0	8	11	43	47	46	0	155
	B	0	0	0	0	6	35	47	32	35	0	155
	C	0	0	0	0	0	17	21	40	28	0	106
	D	0	0	0	0	2	24	40	45	43	53	207
	E	0	0	0	0	10	48	0	41	43	16	158
	F	0	0	0	0	21	35	0	27	40	37	160
	G	0	0	0	0	22	29	0	45	50	32	178
	H	0	0	0	0	8	54	0	40	50	33	185
	I	0	0	0	0	0	24	41	50	40	0	155
	J	0	0	0	0	7	39	54	0	57	52	209
												$\bar{x}$ (± SD) = 167 (± 30)

[1]This value excluded from calculation of the mean due to adult death.

Appendix 5 (Continued)

Neonate Production

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water	Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Day 0	2	5	7	9	12	14	16	19	21	Total
502	A	0	0	0	0	0	12	1	41	42	0	96
	B	0	0	0	0	0	22	40	26	25	0	113
	C	0	0	0	0	0	11	22	27	29	0	89
	D	0	0	0	0	6	25	39	26	41	0	137
	E	0	0	0	0	0	0	0	27	35	0	62
	F	0	0	0	0	0	0	0	10	22	25	57
	G	0	0	0	0	0	4	13	19	25	2	63
	H	0	0	0	0	0	20	35	30	30	0	115
	I	0	0	0	0	0	0	3	6	33	41	83
	J	0	0	0	0	0	10	32	0	8	25	75
												$\bar{x}$ (± SD) = 89 (± 26)
995	A	0	0	0	0	--	--	--	--	--	--	0[1]
	B	0	0	0	0	0	0	0	0	19	16	35
	C	0	0	0	0	0	6	15	9	26	0	56
	D	0	0	0	0	0	4	21	8	18	0	51
	E	0	0	0	0	0	--	--	--	--	--	0[1]
	F	0	0	0	0	0	0	0	0	12	0	12
	G	0	0	0	0	0	5	12	7	4	0	28
	H	0	0	0	0	5	13	19	24	34	0	95
	I	0	0	0	0	0	3	0	5	13	0	21
	J	0	0	0	0	--	--	--	--	--	--	0[1]
												$\bar{x}$ (± SD) = 43 (± 28)

[1]This value excluded from calculation of the mean due to adult death.

Appendix 5 (Continued)

Neonate Production

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water	Well Water

Mean Measured Test Concentration (mg a.i./L)	Replicate	Day 0	2	5	7	9	12	14	16	19	21	Total
1876	A	0	0	0	0	0	--	--	--	--	--	0[1]
	B	0	0	0	0	--	--	--	--	--	--	0[1]
	C	0	0	0	0	0	0	--	--	--	--	0[1]
	D	0	0	0	0	0	--	--	--	--	--	0[1]
	E	0	0	0	0	0	--	--	--	--	--	0[1]
	F	0	0	0	0	0	--	--	--	--	--	0[1]
	G	0	0	0	0	--	--	--	--	--	--	0[1]
	H	0	0	0	0	0	5	0	4	10	5	24
	I	0	0	0	0	0	0	0	4	0	0	4
	J	0	0	0	0	0	0	--	--	--	--	0[1]
												$\bar{x}$ (± SD) = 14 (± 14)

[1]This value excluded from calculation of the mean due to adult death.

Appendix 6

Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Mean Measured Test Concentration (mg a.i./L)	Individual Length (mm)	Mean Length (mm ± SD)	Individual Dry Weights (mg)	Mean Dry Weights (mg ± SD)
Negative Control	4.25	4.18 (± 0.182)	0.7	0.80 (± 0.29)
	4.05		1.0	
	4.30		0.9	
	3.95		0.9	
	4.40		1.4	
	4.30		0.5	
	4.40		0.7	
	4.00		0.6	
	4.00		0.5	
60	4.90	4.89 (± 0.111)	1.7	1.22 (± 0.31)
	4.90		1.3	
	4.90		1.4	
	4.65		1.2	
	4.90		1.4	
	5.00		0.9	
	5.00		0.6	
	4.80		1.4	
	5.00		1.3	
	4.80		1.0	
121	4.75	4.78 (± 0.207)	0.9	1.21 (± 0.34)
	4.80		1.5	
	4.50		1.9	
	4.60		1.6	
	4.85		0.9	
	5.00		1.1	
	4.65		0.9	
	4.90		1.2	
	4.55		1.1	
	5.15		1.0	

Appendix 6 (Continued)

Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Mean Measured Test Concentration (mg a.i./L)	Individual Length (mm)	Mean Length (mm ± SD)	Individual Dry Weights (mg)	Mean Dry Weights (mg ± SD)
247	4.75	4.77 (± 0.206)	1.0	0.96 (± 0.16)
	4.75		1.2	
	4.60		1.1	
	4.65		0.8	
	5.00		0.9	
	4.35		0.8	
	4.80		0.8	
	5.00		0.9	
	4.75		1.2	
	5.00		0.9	
502	4.35	4.26 (± 0.480)	1.0	0.88 (± 0.16)
	4.40		0.9	
	4.30		1.0	
	4.25		1.1	
	4.45		1.0	
	2.95		0.6	
	4.20		0.9	
	4.65		0.9	
	4.60		0.7	
	4.40		0.7	
995	4.10	3.69 (±0.352)	0.7	0.51 (±0.16)
	3.65		0.7	
	3.60		0.4	
	3.30		0.4	
	3.65		0.3	
	4.20		0.6	
	3.30		0.5	
1876	2.75	2.68 (±0.106)	0.1	0.10 (±0.0)
	2.60		0.1	

Appendix 7

Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add the experimental start and termination dates, test concentrations and test substance number.
2. The protocol was amended to correct the analytical sampling schedule.
3. During the second-generation exposure, neonates were observed at 2, 24 and 48 hours.
4. Treatment groups were indiscriminately positioned.
5. There were two instances where the continuous temperature recorder reached 18°C.

Appendix 8

Personnel Involved in the Study

The following key Wildlife International Ltd. personnel were involved in the conduct or management of this study:

1. Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
2. Willard B. Nixon, Ph.D., Director, Analytical Chemistry
3. Raymond L. Van Hoven, Ph.D., Scientist
4. Cary A. Sutherland, Laboratory Supervisor
5. Kurt R. Drottar, Senior Biologist
6. Susan T. Platania, Biologist